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(54) Title: **USE OF ONE OR MORE PEPTIDES IN A COSMETIC COMPOSITION OR FOR THE PREPARATION OF A MEDICAMENT**

(57) Abstract [English, as published.]

The invention discloses the use, as an active ingredient in a physiologically acceptable medium, in a cosmetic composition or for the preparation of a medicament, of an effective amount of one or more peptides containing the tripeptide Lysine-Proline-Valine, or of any functional biological equivalent thereof, wherein the Proline residue is in the form of its dextrogyral optical isomer, for treating inflammations. A cosmetic treatment method is also described.

[list of state abbreviations on next page is omitted]

USE OF ONE OR MORE PEPTIDES IN A COSMETIC COMPOSITION OR FOR THE PREPARATION OF A MEDICAMENT.

The present invention concerns the use, as an active ingredient in a physiologically acceptable medium, in a cosmetic composition or for the preparation of a medicament, of an effective amount of at least one peptide containing the tripeptide Lysine-Proline-Valine, or of any functional biological equivalent thereof, wherein the Proline residue is in the form of its dextrogyral optical isomer, for treating inflammations.

Inflammation is a group of biological reactions found in all levels of animals. In humans, two out of three diseased individuals present an inflammatory syndrome. The inflammation may be localized.

It may be defined as the first response to any local injury through a series of nonspecific reactions triggered regardless of the initial cause and occurring in three stages: vascular, cellular vascular, and tissue fibrosis.

Swelling, pain, redness, and heat are terms that may be used to describe localized inflammation. These are usually due to the infiltration of the injured tissues by edema and/or capillary vasodilatation.

The signs of inflammation may range to fever, a condition of general malaise, and/or an increase in the concentration of certain blood plasma proteins.

This is a phenomenon which involves, among other things, a series of local cellular reactions and the release of cytokines and other mediators such as substance P, prostaglandins, histamine, or even serotonin.

It manifests in a modification of blood flow with, at the site of injury, an increase in vascular permeability resulting in an escape of plasma proteins and of cells to the extracellular fluid as well as extravasion of leukocytes, primarily of neutrophilic leukocytes and macrophages toward the inflammatory site.

These phenomena are, in fact, the result of the action of the mediators of the inflammation.

Among the factors involved in these inflammatory phenomena, it is possible to mention the cytokines, including, in particular, interleukin 1-α, interleukin 1-β, interleukin 6, tumor necrosis factors α and β (TNF-α and -β, the chemokines, such as interleukin 8, or the monocyte chemotactic activating factor (MCAF), or other chemotactic factors responsible for recruiting lymphocytic, monocytic, Langerhans', or basophilic cells at the level of the inflammatory site, such as leukotrienes B-4, or even other factors involved in the inflammatory cascade, such as arachidonic acid, or the prostaglandins, including especially the E2 prostaglandins.

The inflammatory phenomena are associated with numerous pathologies.
Examples which can be cited are erythema solare, pruritus, erythema nodosum, urticaria, systemic mastocytosis, psoriasis, insect bites, other dermatologic disorders such as atrophic polychondritis, erythermalgia, and necrobiosis lipoidica. Disseminated lupus erythematosus, spondyloarthropathies, or joint disorders of chronic enteropathies may also be mentioned.

For many years, the pharmaceutical industry has been seeking substances enabling treatment of inflammation.

In this regard, it has recently been proposed to use a sufficient quantity of a derivative of the melanocyte-stimulating hormone of type α (α-MSH) or melanotropin and especially the peptide containing the tripeptide Lysine-Proline-Valine (US 5,028,592, US 5,157,023).
It has, however, been shown that the optical form of the isomers used in the composition of the tripeptide was very significant. Thus, it has been shown that when the Proline residue appears in the tripeptide in its dextrogyral optical isomer form (DPro), the tripeptide or the peptide containing the tripeptide lost all efficacy in the treatment of inflammation (Hiltz et al. Peptides, vol. 12, pp. 767-771, 1991).

The applicant has now discovered, after significant research into the question, that a peptide containing the tripeptide Lysine-Proline-Valine, in which the Proline residue appears in the tripeptide in its dextrogyral optical isomer form (DPro), or any functional biological equivalent thereof, is active in the treatment of inflammation.
The expression "functional biological equivalent" means a peptide functionally equivalent in terms of biological function of which at least one of the amino acid residues can have been replaced by an amino acid residue with a similar hydropathic index.

This discovery is the foundation of the present invention.

Thus, the invention concerns the use, in a cosmetic composition and/or for the preparation of a medicament to treat inflammation, of at least one peptide containing the tripeptide Lysine-Proline-Valine, in which the Proline residue appears in its dextrogyral optical isomer form (DPro), or any functional biological equivalent thereof.

In the area of the amino acids, the geometry of the molecules is such that they can theoretically appear as different optical isomers. There is, in effect, a molecular shape of the amino acid (aa) such that it rotates the plane of polarized light to the right (dextrogyral shape or D-aa), and a molecular shape of the amino acid (aa) such that it rotates the plane of polarized light to the left (levogyral shape or L-aa).

Nature selected only the levogyral shape for natural amino acids. Consequently, a peptide of natural origin will consist of only type L-aa amino acids.
However, chemical synthesis in the lab enables preparation of amino acids with both possible shapes. Starting from this basic material, is possible, at the time of synthesis of peptides, to incorporate amino acids in both the levogyral and the dextrogyral optical isomer form.
Thus, it is possible, at the time of peptide synthesis, to incorporate, not only the D-Proline (D-Pro), but also Lysine or Valine amino acid residues indifferently in their form D-Lysine (D-Lys), L-Lysine (L-Lys), D-Valine (D-Val), or L-Valine (L-Val).

Thus, the invention concerns more specifically the use of a sufficient quantity of the peptide as defined above, characterized in that the Lysine or Valine residues of the tripeptide Lysine-(D) Proline-Valine constituting the peptide are indifferently in the form of dextrogyral or levogyral optical isomers.

Thus, it is possible to mention the peptides containing at least one of the following tripeptides:
D-Lys-D-Pro-D-Val,
D-Lys-D-Pro-L-Val,
L-Lys-D-Pro-D-Val,
L-Lys-D-Pro-L-Val.

The tripeptide is advantageously located at the C-terminal end of the peptide.

Preferably, the peptide used according to the invention is the tripeptide Lysine-Proline-Valine in which the Proline residue appears in its dextrogyral optical isomer form (DPro).

Also preferably, the peptide used according to the invention contains the tripeptide Lysine-Proline-Valine in which the Lysine, Proline, and Valine residues appear in the form of dextrogyral optical isomers (DLys-DPro-DVal).

According to the invention, it is, of course, possible to use more than one peptide. In this case, the peptide mixture may consist of one of the possible combinations of the peptides described above.

In the text which follows, in general, the term "Proline" is understood to mean the Proline residue in its dextrogyral optical isomer form (DPro) and the term "peptide" refers to both the "peptide containing the tripeptide Lysine-Proline-Valine, or any functional biological equivalent thereof", and the isolated "tripeptide Lysine-Proline-Valine", in which the Proline residue is in its dextrogyral optical isomer form (DPro).

It is possible that, for reasons of resistance to degradation, it may be necessary to use, according to the invention, a protected form of the peptide. The form of the protection must obviously be a biologically compatible form. Numerous forms of biologically compatible protections can be envisaged, such as, acylation or acetylation of the amino-terminal end or amidation of the carboxy-terminal end.

Thus, the invention concerns a use as previously defined, characterized in that the peptide is in either a protected form or an unprotected form.

Preferably, according to the invention a protection based on either acylation or acetylation of the amino-terminal end or on amidation of the carboxy-terminal end or on both is used.

The term "effective quantity of the active ingredient" means the quantity necessary to attain the desired result.

More specifically, in the cosmetic composition, the peptide is present in a quantity such that the tripeptide Lysine-Proline-Valine is at a concentration between 10^{-12} M and 10^{-3} M, and preferably between 10^{-9} M and 10^{-4} M.

More specifically, in the preparation of a medicament, the peptide is present in a quantity such that the tripeptide Lysine-Proline-Valine can be used at a concentration between 10^{-12} M and 1 M, and preferably between 10^{-6} M and 10^{-1} M.

It is clear that the person skilled in the art knows how to adjust this quantity of material according to whether he uses the peptide containing the tripeptide Lysine-Proline-Valine, or any functional biological equivalent thereof or the tripeptide Lysine-Proline-Valine.

The composition according to the invention may be administered parenterally, enterally, or even topically. Preferably, the composition is administered topically.

The physiologically acceptable medium in which the peptide is used according to the invention can be anhydrous or aqueous. The expression "anhydrous medium" means a solvent medium containing less than 1 % water. This medium may consist of a solvent or mixture of solvents selected more specifically from among the low alcohols C_2-C_4, such as ethyl alcohol, the alkylene glycols such as propylene glycol, and the alkyl ethers of alkylene glycols or of dialkylene glycols, including the alkyl or alkylene radicals containing from 1 to 4 carbon atoms. The expression "aqueous medium" means a medium consisting of water or a mixture of water and of another physiologically acceptable solvent, selected, in particular, from among the above mentioned organic solvents. In this latter case, these other solvents, when present, represent approximately 5 to 95 % by weight of the composition.

It is possible that the physiologically acceptable medium can contain other adjuvants conventionally used in cosmetics or pharmaceuticals, such as surfactants, thickeners or gelatinizers, cosmetic agents, preservatives, alkalinizers or acidifiers well known in the art, and in sufficient quantities to obtain the form of presentation desired, especially as a more or less thick lotion, a gel, an emulsion, or a cream. The use may possibly be implemented in a pressurized form in aerosol or vaporized from a spray bottle.

It is also possible to use, in association with the peptide, compounds already described for their anti-inflammatory activity.

Specifically, glucocorticoids, vitamin D and its derivatives, and nonsteroid anti-inflammatories may be mentioned.

The use of the peptide according to the invention may be implemented by topical application of a cosmetic composition containing an effective quantity of at least one peptide containing the tripeptide Lysine-Proline-Valine in which the Proline residue appears in its dextrogyral optical isomer form (DPro) to a part of the body presenting symptoms of inflammation.

Thus, the present invention has the further object of a method of cosmetic treatment, characterized in that a cosmetic composition containing an effective quantity of at least one peptide containing the tripeptide Lysine-Proline-Valine in which the Proline residue appears in its dextrogyral optical isomer form (DPro) is applied on the skin, on the scalp, and/or on the mucosas of cutaneous zones presenting symptoms of inflammation.

The method of cosmetic treatment of the invention may be implemented, in particular by applying the cosmetic compositions as defined above according to the customary technique for use of these compositions. For example: application of creams, gels, sera, lotions, makeup removal lotions, or sunscreen compositions on the skin or shampoos on the scalp, or toothpaste on the gums.

The following examples are provided by way of illustration and in no way limit the scope of the invention. The proportions indicated in the compositions are weight-percentages of the total weight of the composition.

Example 1: Dose-response activity of the tripeptide Ac - LPV - NH_2* on the production of interleukin 1α in the surnatant of hairs plucked from a patient with inflammatory alopecia:

Plucked hairs are collected from the top of the head of a volunteer with inflammatory alopecia. They are placed in a Williams' E survival medium (sold by the company Gibco BRL) containing penicillin G (100 units/ml), streptomycin-S (100 µg/ml), amphotericin (250 ng/ml), in the presence or absence (control) of the tripeptide Ac - LPV - NH_2* synthesized to order by the company Neosystem S.A. (Strasbourg) at the doses indicated. After 20 hours of incubation, the culture surnatants are collected in a tube, then centrifuged for 5 minutes at 14,000 rpm (Eppendorff centrifuge, model 5415C). The surnatants are then collected in a clean tube and placed at 4°C.
The interleukin 1-α concentration is then evaluated for 100 µl of the surnatant using a Biotrak ELISA kit marketed by the company Amersham, following the supplier's instructions.

Results:

	Dose	IL-1α	% inhibition
Control		21.8 pg/ml	
Ac - LPV - NH_2*	10 µM	6.1 pg/ml	72 %
Ac - LPV - NH_2*	1 µM	11.1 pg/ml	49 %

Example 2:

Inhibition of the expression of mRNAs of pro-inflammatory and inflammatory cytokines in response to the tripeptide Ac - LPV - NH_2*.

Ten plucked hairs are collected from the top of the head of a volunteer with inflammatory alopecia. They are placed in a Williams' E survival medium (sold by the company Gibco BRL) containing penicillin G (100 units/ml), streptomycin-S (100 µg/ml), amphotericin (250 ng/ml), in the presence (treated batch) or absence (control batch) of the tripeptide Ac - LPV - NH_2* synthesized to order by the company Neosystem S.A., Strasbourg.

After 3.5 hours of incubation, the mRNAs corresponding to these two batches of hair are purified using a kit "quick prep mRNA purification kit" marketed by the company Pharmacia. Complementary DNAs of these mRNAs are then prepared using a reverse transcription kit marketed by the company Pharmacia following the supplier's instructions, then subjected to a polymerization chain reaction (PCR) step using specific primers of the mRNAs of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), of IL-1alpha, of the receptor of IL-1 of type 1, and of the receptor of IL-1 of type 2. The quantities of DNA amplified are then evaluated by electrophoresis on agarose gel at 1.5 % in the presence of ethidium bromide. The intensity of the bands is assessed under ultraviolet radiation using a video camera and analytical software (Bioprofil TM) marketed by the company Vilbert-Lourmat. The intensity of the bands obtained with the primers IL-1α, IL-1R1, IL-1R2 is divided by the intensity of the bands obtained with the primers amplifying the internal standard GAPDH.

Results:

% of expression	IL-1α	IL-1R1	IL-1R2
Control batch	100 %	100 %	100 %
Treated batch	0 %	26 %	21 %

Example 3:

Inhibiting effect of Ac - LPV - NH_2* on the expression of mRNAs of the IL-1α calculation of the ratio IL-1α /GAPDH:

Five hairs are plucked from two different donors, then incubated for 20 hours at 37°C (5 % CO2) in Williams' E medium supplemented with antibiotics, with glutamine, and in the presence of Ac - LPV - NH_2* at the concentrations indicated. The control has no peptide added. The results are expressed in % of the control.

Donors		A	B
Control		100 %	100 %
Ac - LPV - NH_2*	10 µM	28 %	28.7 %
Ac - LPV - NH_2*	1 µM	51 %	62 %
Ac - LPV - NH_2*	0.1 µM	ND	53 %

ND: Not determined

Example 4:

Measure of the inhibition of the production of PGE2 by cells in the catagenic papilla in in-vitro culture:

The cells (1000 per well), at pass 24, are incubated in medium 199 marketed by the company GIBCO in the presence of 1 % fetal bovine serum and antibiotics. 20 hours later, the medium is

replaced by an identical medium, but containing the tripeptides to be evaluated at the final concentration of 10 μM. 5 hours later, the interleukin 1α is added at the final concentration of 10 ng/ml. 20 hours later, the rates of PGE2 produced by the papilla cells in culture are evaluated using a Biotrak kit marketed by the company Amersham, following the supplier's instructions. This method thus enables evaluating the inhibiting effect of these tripeptides on the production of PGE2 induced by a pro-inflammatory cytokine: interleukin 1α.

	Dose	PGE2 (pg/ml)	
Control		9.2	
IL-1α		100.2	
			% inhibition
Ac - LPV - NH_2*	10 μM	22.0	78 %
Ac- L-P-V-NH_2**	10 μM	10.0	90 %
Ac-L-(D)P-V-NH_2***	10 μM	24.6	75 %

These results indicate, surprisingly, a comparable anti-inflammatory capacity of the tripeptides containing either the form (D)-Pro or the natural form (L)Pro.

Example 5: Examples of compositions containing the tripeptide Ac - LPV - NH_2*. These compositions are obtained by conventional preparation techniques, in particular, by simple mixing of the ingredients.

Composition 1: Spray:

Ac - LPV - NH_2*	5×10^{-5}	g
Minoxidil	0.5	g
Ethanol at 95°	55.1	g
Propylene glycol	22.8	g
Perfume	qs	

Demineralized water	qsp	100	g

Composition 2: Day lotion:

Ac - LPV - NH2*		12.5×10^{-6}	g
2,4 diaminopyrimidine-3-oxide		0.75	g
Ethanol at 95°		30	g
Perfume		qs	
Dyes		qs	
Demineralized water	qsp	100	g

Composition 3: Liposome gel:

Natipide II® (i.e., 2 g in phospholipids)		10	g
Ac - LPV - NH_2*		5×10^{-5}	g
Carbomer		0.25	g
Triethanolamine		qs pH = 7	
Preservatives		qs	
Demineralized water	qsp	100	g

®Mixture Water/Alcohol/Lecithin from the company Nattermann

Composition 4: Niosome gel:

Chimexane NS®		1.8	g
Monosodium stearoylglutamate		0.2	g
Ac - LPV - NH_2*		7.5×10^{-4}	g
Carbomer		0.2	g
Triethanolamine		qs pH = 7	

Preservatives		qs	
Perfumes		qs	
Demineralized water	qsp	100	g

®Nonionic surfactant sold by the company Chimex.

Composition 5: Niosome lotion:

Chimexane NL®		0.475	g
Cholesterol		0.475	g
Monosodium stearoylglutamate		0.05	g
Ac-LPV-NH_2*		10^{-3}	g
Preservatives		qs	
Dyes		qs	
Perfume		qs	
Demineralized water	qsp	100	g

®Nonionic surfactant sold by the company Chimex.

Composition 6: Protective cream: O/E emulsion

Cetylstearylic alcohol/cetylstearylic alcohol oxyethylenated with 33 moles of oxyethylene (80/20)		5	g
Monostearate of glycerol		1.5	g
Cetyl alcohol		0.75	g
Vaseline oil		10	g
Polydimethylsiloxane		0.75	g
Glycerin		4	g
Preservatives		qs	
Ac-LPV-NH_2*		5 × 10-3	g
Demineralized water	qsp	100	g

Composition 7: Intradermally injectable solution

Ac-LPV-NH_2*		0.7	mg
Physiologic serum			
(NaCl 9g/H2O qsp 100 ml)	qsp	1	ml

*: Acetyl - (D)Lys - (D)Pro - (D)Val - NH_2

**: Acetyl - (L)Lys - (L)Pro - (L)Val - NH_2

***: Acetyl - (L)Lys - (D)Pro - (L)Val - NH_2

CLAIMS

1. Use in a cosmetic composition or for the preparation of a medicament, of an effective quantity of at least one peptide containing the tripeptide Lysine-Proline-Valine wherein the Proline residue appears in its dextrogyral optical isomer form (DPro) or of any functional biological equivalent thereof, to treat information.

2. Use according to the preceding claim, characterized in that the tripeptide is located at the C-terminal end of the peptide.

3. Use according to one of the preceding claims, characterized in that the peptide is the tripeptide Lysine-Proline-Valine wherein the Proline residue appears in its dextrogyral optical isomer form (DPro).

4. Use according to one of the preceding claims, characterized in that the peptide is the tripeptide Lysine-Proline-Valine wherein the Lysine, Proline, and Valine residues appear in the form of dextrogyral optical isomers (D-Lys-D-Pro-D-Val).

5. Use according to one of the preceding claims, characterized in that the peptide is or is not in a protected form.

6. Use according to the preceding claim, characterized in that the protection consists of protection based either on acylation or acetylation of the amino-terminal end or on amidation of the carboxy-terminal end or on both.

7. Use in a cosmetic composition according to any one of the preceding claims, characterized in that the tripeptide Lysine-Proline-Valine wherein the Proline residue appears in its dextrogyral optical isomer form (DPro) is used at a concentration between 10^{-12} M and 10^{-3} M and preferably between 10^{-9} M and 10^{-4} M.

8. Use for the preparation of a medicament according to any one of the claims 1 through 6, characterized in that the tripeptide Lysine-Proline-Valine wherein the Proline residue appears in its dextrogyral optical isomer form (DPro) is used at a concentration between 10^{-12} M and 1 M and preferably between 10^{-6} M and 10^{-1} M.

9. Method of cosmetic treatment, characterized in that a cosmetic composition containing an effective quantity of at least one peptide containing the tripeptide Lysine-Proline-Valine wherein the Proline residue appears in its dextrogyral optical isomer form (DPro) is applied on the skin, on the scalp, and/or on the mucosas presenting symptoms of inflammation.

[International search report was provided in matching English and French.]